THE NITROGENOUS METABOLISM OF BACILLUS PROTEUS

STUDIES IN BACTERIAL METABOLISM. LXV

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One of the most conspicuous, versatile and puzzling groups of bacteria commonly encountered is that one on which Hauser [1] conferred the very appropriate appellation "Proteus." Originally, the term "Proteus" seems to have referred to four varieties or members of a peculiar morphologic type of bacillus which differed quantitatively in their respective abilities to induce liquefaction in gelatin mediums. Later and more intensive studies have revealed other peculiarities which emphasize especially the apparent heterogenicity of the immunologic armamentarium of at least certain strains of the type organism. Thus Frost [2] has isolated a strain of B. proteus which is agglutinated in high dilution with a specific typhoid serum. Felix and Weil [3] have shown that at least two strains of B. proteus [4] (X_2 and X_{19}) will agglutinate with serums from a majority of typhus fever patients. It is significant that the latter strains are distinctly less active proteolytically than the ordinary, freshly isolated varieties of the Hauser type, designated by him Proteus vulgaris.

Many observers have failed to recognize the striking proteolytic activity of typical strains of the vulgaris type. Berman and Rettger,[5] criticising Kendall and Walker's [6] work on the influence of utilizable carbohydrate on the appearance of an active, soluble proteolytic enzyme of B. proteus, say: "A bacterial proteolytic enzyme, as a rule, is not produced within the first twenty-four hours, but requires a longer period before it makes its appearance in detectable quantities."

It is quite evident that Berman and Rettger were not familiar with the more typical and active strains of B. proteus, as will be shown

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[1] Weber: Fäulnisbakterien und deren Beziehungen zur Septikäme, 1885.
[2] Public Health and Marine Hospital Laboratory, Bull. 66, 1910, Washington, D. C.
[3] Wien. klin. Wchnschr., 1916, 29, pp. 873, 974.
[4] Bengston: Public Health Reports, 1919, 34, p. 2446.
[5] Jour. Bacteriol., 1918, 3, p. 389.
[6] Jour. Infect. Dis., 1915, 17, p. 442.

later. Wolf[7] also states that cultures of B. proteus do not induce that rapid liquefaction of gelatin mediums which have been ascribed to them. It seems not improbable from a perusal of this article that Wolf was working with one of the two cultures of B. proteus, X_2 or X_{19}, which agglutinate with typhus serums. These strains, as Miss Bengston[4] has shown, are rather indolent in their gelatin liquefying powers.

The question naturally presents itself: What is Bacillus proteus? Flügge[8] classifies the members of the group primarily on their respective abilities to liquefy gelatin—Proteus vulgaris being the most active, Proteus mirabilis less so, and Proteus zenkeri quite without liquefying powers. In this connection, it will be remembered that the ability of strains of B. proteus (Proteus vulgaris) to induce liquefaction in gelatin is frequently decreased or lost through prolonged development in artificial mediums. Also, the saccharose-fermenting power may be lost on prolonged cultivation. A strain that has lost its gelatin-liquefying and saccharose-fermenting powers assumes superficially many of the cultural characteristics of the Morgan bacillus.[9] Theobald Smith[10] made the highly important observation that Proteus vulgaris ferments glucose and saccharose with the production of gas and acid, lactose being unattacked. It is now known that mannitol also is unfermented. A point of some importance also is the inability of a majority, if indeed not of all, of the true members of the Proteus vulgaris type to ferment mannose.

A typical strain of B. proteus (Proteus vulgaris), therefore, induces a rapid liquefaction of gelatin by means of a soluble, or exo-enzyme. It ferments glucose and saccharose with the formation of gas and acid, but fails to ferment lactose, mannose or mannitol. A restriction or loss of gelatin-liquefying power and the loss of saccharose-fermenting power are rather common modifications of cultural and chemical properties of the typical organism. The association of Proteus vulgaris with human lesions is accompanied frequently by one or more of these modifications.

The study of the nitrogenous metabolism of B. proteus reported in the following was undertaken to determine the changes induced in nitrogenous constituents of a gelatin-peptone-meat juice medium by an active culture of the organism after various periods of incubation. The

[7] Jour. Path. & Bacteriol., 1919, 22, p. 284.
[8] Die Mikroorganismen, 1896, 2, p. 272.
[9] Morgan: British Med. Jour., 1906, 1, p. 908.
[10] The Fermentation Tube, Wilder Quarter Century Book, 1893, p. 213.

medium contained the usual ingredients, including 5% of gelatin and 1% of peptone, made up in meat infusion. The reaction at the start was slightly alkaline—P_H 7.3 to be exact. The cultures were studied at various intervals, and, also, an effort was made to measure the activity

TABLE 1

B. PROTEUS

Proteus No. 1 June 28, 1921 Cultures	Day	Control	Plain	Percentage of Glucose						
				0.1	0.2	0.3	0.4	0.5	0.75	1.5
Total nitrogen	1	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001	
Protein nitrogen		0.721	0.531	0.564	0.598	0.609	0.665	0.676	0.721	
Nonprotein nitrogen		0.280	0.470	0.437	0.403	0.392	0.336	0.325	0.280	
Polypeptid nitrogen		0.209	0.389	0.361	0.330	0.319	0.265	0.253	0.213	
Amino nitrogen		0.039	0.032	0.028	0.028	0.029	0.030	0.032	0.029	
Ammonia nitrogen		0.032	0.049	0.048	0.045	0.044	0.041	0.040	0.038	
Total nitrogen	2	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001
Protein nitrogen		0.721	0.262	0.195	0.385	0.419	0.453	0.497	0.497	0.743
Nonprotein nitrogen		0.280	0.739	0.806	0.616	0.582	0.548	0.504	0.504	0.258
Polypeptid nitrogen		0.209	0.638	0.709	0.527	0.415	0.475	0.429	0.434	0.191
Amino nitrogen		0.039	0.032	0.030	0.029	0.029	0.028	0.027	0.026	0.030
Ammonia nitrogen		0.032	0.069	0.067	0.060	0.048	0.045	0.048	0.044	0.037
Total nitrogen	3	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001	
Protein nitrogen		0.721	0.239	0.239	0.284	0.352	0.430	0.453	0.531	
Nonprotein nitrogen		0.280	0.762	0.762	0.717	0.649	0.571	0.548	0.470	
Polypeptid nitrogen		0.209	0.647	0.647	0.608	0.536	0.473	0.469	0.400	
Amino nitrogen		0.039	0.036	0.036	0.032	0.030	0.047	0.030	0.028	
Ammonia nitrogen		0.032	0.089	0.089	0.077	0.056	0.051	0.049	0.042	
Total nitrogen	4	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001	
Protein nitrogen		0.721	0.060	0.073	0.139	0.228	0.262	0.307	0.351	
Nonprotein nitrogen		0.280	0.941	0.928	0.862	0.773	0.739	0.694	0.650	
Polypeptid nitrogen		0.209	0.796	0.777	0.709	0.696	0.654	0.613	0.571	
Amino nitrogen		0.039	0.028	0.035	0.035	0.018	0.035	0 032	0.030	
Ammonia nitrogen		0.032	0.117	0.116	0.118	0.059	0.050	0.049	0.049	
Total nitrogen	5	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001
Protein nitrogen		0.721	0.161	0.127	0.161	0.206	0.206	0.239	0.340	0 822
Nonprotein nitrogen		0.280	0.840	0.847	0.840	0.795	0.795	0.762	0.661	0.179
Polypeptid nitrogen		0.209	0.663	0.694	0.679	0.654	0.667	0.648	0.567	0.103
Amino nitrogen		0.039	0.033	0.031	0.032	0.024	0.025	0.035	0.030	0.039
Ammonia nitrogen		0.032	0.144	0.149	0.129	0.117	0.103	0.079	0.064	0.037
Total nitrogen	7	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001
Protein nitrogen		0.721	0.038	0.094	0.071	0.060	0.083	0.094	0.105	0.732
Nonprotein nitrogen		0.280	0.963	0.907	0.930	0.941	0.918	0.907	0.896	0.269
Polypeptid nitrogen		0.209	0.696	0.687	0.703	0.757	0.750	0.730	0.722	0.185
Amino nitrogen		0.039	0.036	0.025	0.032	0.035	0.042	0.040	0.033	0.044
Ammonia nitrogen		0.033	0.204	0.195	0.195	0.149	0.126	0.137	0.141	0.039
Total nitrogen	14	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001	
Protein nitrogen		0.721	0.162	0.151	0.140	0.218	0.151	0.106	0.106	
Nonprotein nitrogen		0.280	0.839	0.850	0.861	0.783	0.850	0.895	0.895	
Polypeptid nitrogen		0.209	0.587	0.577	0.566	0.511	0.580	0.600	0.624	
Amino nitrogen		0.039	0.039	0.026	0.030	0.059	0.057	0.079	0.050	
Ammonia nitrogen		0.033	0.213	0.247	0.265	0.213	0.213	0.216	0.221	

of the soluble proteolytic enzyme at the same periods. The methods have been described previously.[6,11,12] The results are shown in the tables.

[11] Kendall: Study LVII, Jour. Infect. Dis., 1922, 30, p. 211.

[12] Kendall and Bly: Ibid., Study LXII.

DISCUSSION ON TABLE 1

Cultures: The salient features of the changes in the nitrogenous constituents of the various gelatin-glucose-mediums are indicated clearly by the analytic figures, which express the results of duplicate determinations in terms of milligrams of nitrogen per 100 c c of culture. The most striking change is that of the protein nitrogen fraction, which even within 24 hours of incubation has diminished from 72% (that of the uninoculated control) to 53% in the glucose-free gelatin. It must be remembered that this remarkable decrease in protein nitrogen, due to the activity of the soluble enzyme of the organism, is somewhat greater than the figures indicate because there is produced simultaneously some increase in this fraction due to the formation of bacterial protein.[13]

There is coincidently a not inconsiderable increase in ammonia nitrogen, greater in the glucose-free and 0.1% glucose gelatin, less in the mediums containing larger proportions of sugar. The increase in ammonia from the first day of incubation, when it amounts to less than 2%, to the second week of incubation when it reaches almost 20%, is an indication of the deaminizing activity of the microbe. It is a striking fact that the amino-nitrogen fraction shows little increase during this time. Apparently the significant change is a degradation of the original protein nitrogen directly to the nonprotein-nitrogen fraction, and specifically to that residual nonprotein nitrogen fraction which for convenience of discussion is termed "polypeptid" nitrogen.[11] This change amounts to an increase of "polypeptid" nitrogen from about 20% in the uninoculated control to over 70% in the 7-day culture in plain and low percentage glucose mediums, as is clearly shown in the tables.

In concentrations of glucose exceeding 0.3%, the protein-nitrogen fraction, even after several days' incubation, fails to reach quite as low a level as in the plain gelatin cultures and those containing lesser amounts of glucose. The suggestion is made, which has considerable evidence in its favor, that the amount of bacterial protein is somewhat greater in the higher glucose concentrations; in other words, bacterial development has proceeded further. Filtration of the bacteria from one such culture reduced the protein nitrogen of the filtrate from 10% to

[13] Compare Bacillus dysenteriae (Study LVIII), typhosus (Study LVII), and Studies LX to LXIV, inclusive, for quantitative measurements of the increase in protein nitrogen due to the growth of nonliquefying bacteria.

6% of the original protein nitrogen.[14] The addition of 1.5% glucose to the gelatin medium effectively prevented evidences of proteolysis. The organisms apparently failed to ferment all the glucose, presumably because the accumulation of acid products of fermentation prevented the growth of the bacteria after a few days' incubation. With the progressive increase in glucose content the delay in ammonia formation is clearly outlined.

Berman and Rettger,[5] criticising Kendall and Walker's study on the influence of glucose on the metabolism of B. proteus, state: "The present investigation has shown conclusively that fermentable sugars in moderate amounts do not affect the nitrogen metabolism of bacteria—under conditions of favorable environment." Their contention is based on the study of cultures of B. proteus and other bacteria using the method of formol titration of Sörensen and the biuret test of Vernon as measures of nitrogenous change. Their tests were made in two kinds of mediums, one containing 0.5% peptone, 0.25% beef extract, 0.5% NaCl, and 0.2 and 0.4% glucose, respectively, and the same mediums containing in addition 0.5% K_2HPO_4. This is a large amount of phosphoric acid in proportion to the other constituents of the medium. Their results are puzzling.

In the control medium, containing 0.2% glucose and 0.5% K_2HPO_4, they record sugar as absent; in each culture of B. proteus in this medium, however, sugar is invariably recorded as "present." It is very significant, as Jones[15] has pointed out, that in the 0.2% glucose medium without the K_2HPO_4, sugar is reported "absent" after 24 hours' incubation. It would appear from their own tables that Berman and Rettger's conclusions relative to the influence of small amounts of utilizable sugar on the metabolism of B. proteus are incorrect; unless their tables contain unrecognized typographical errors. Perhaps the most striking instance of such incompatibility is the reported absence of sugar in the control phosphate—0.2% glucose medium on page 397 of their article.

The nitrogenous metabolism of a culture of B. proteus in the gelatin-peptone-meat infusion medium described in the foregoing, with graded amounts of glucose from 0.1% to 1.5%, shows therefore several distinctive features: The sparing action of utilizable carbohydrate in sufficient amounts to provide more than the organism can completely utilize is shown clearly in the column in which the nitrogenous change in this medium is recorded. Smaller amounts of glucose in descending order retard the rate of deamination by the bacilli and, also, as the table shows clearly, retard in regular order, but do not ultimately prevent, the decrease in protein nitrogen and the corresponding increase in "polypeptid" nitrogen.

[14] This experiment was made on the 7-day culture in 0.75 glucose gelatin. Before filtration the protein nitrogen was 105 mg. per 100 c c culture medium. After filtration the protein nitrogen was only 61 mg. per 100 c c culture medium.

[15] Journal Infect. Diseases, 1920, 27, 169.

The question naturally arises: What proportion of the decrease in protein nitrogen, and therefore what proportion of the increase in the "polypeptid" nitrogen is due to the soluble enzyme of the bacteria, and what part is due to the intracellular nitrogenous metabolism of the bacteria, respectively?

TABLE 2

B. PROTEUS

Carbol Gelatin Enzyme	Day	Control	Plain	Percentage of Glucose						
				0.1	0.2	0.3	0.4	0.5	0.75	1.5
Total nitrogen	1	0.672	0.672	0.672	0.672	0.672	0.672	0.672	0.672	
Protein nitrogen		0 542	0.325	0.280	0.381	0.392	0.426	0.426	0.471	
Nonprotein nitrogen		0.130	0.347	0.392	0.291	0.280	0.246	0.246	0.201	
Polypeptid nitrogen		0.102	0.314	0.360	0.257	0.252	0.217	0.217	0.172	
Amino nitrogen		0.020	0.025	0.024	0.028	0.022	0.023	0.023	0.023	
Ammonia nitrogen		0.008	0.008	0.008	0.006	0.006	0.006	0.006	0.006	
Total nitrogen	2	0.672	0.672	0.672	0.672	0.672	0.672	0.672	0.672	0.672
Protein nitrogen		0.542	0.235	0.246	0.314	0.403	0.414	0.358	0.370	0.515
Nonprotein nitrogen		0.130	0.437	0.426	0.358	0.269	0.258	0.314	0.302	0.157
Polypeptid nitrogen		0.098	0.384	0.379	0.314	0.235	0.227	0.285	0.273	0.125
Amino nitrogen		0.023	0.044	0.038	0.035	0.026	0.022	0.022	0.020	0.023
Ammonia nitrogen		0.009	0.009	0.009	0.008	0.008	0.009	0.007	0.009	0.009
Total nitrogen	3	0.672	0.672	0.672	0.672	0.672	0.672	0.672	0.672	
Protein nitrogen		0.542	0.078	0.112	0.146	0.202	0.258	0.291	0.347	
Nonprotein nitrogen		0.130	0.594	0.560	0.526	0.470	0.414	0.381	0.325	
Polypeptid nitrogen		0.097	0.534	0.498	0.475	0.430	0.368	0.344	0.294	
Amino nitrogen		0.023	0.048	0.052	0.042	0.031	0.038	0.029	0.023	
Ammonia nitrogen		0.010	0.012	0.010	0.009	0.009	0.008	0.008	0.008	
Total nitrogen	4	1.330	1.330	1.330	1.330	1.330	1.330	1.330	1.330	
Protein nitrogen		1.061	0.389	0.447	0.524	0.515	0.793	0.793	0.804	
Nonprotein nitrogen		0.269	0.941	0.883	0.806	0.815	0.537	0.537	0.526	
Polypeptid nitrogen		0.119	0.860	0.819	0.737	0.459	0.484	0.484	0.478	
Amino nitrogen		0.035	0.065	0.048	0.053	0.041	0.038	0.037	0.032	
Ammonia nitrogen		0.015	0.016	0.016	0.016	0.015	0.015	0.016	0.016	
Total nitrogen	5	1.330	1.330	1.330	1.330	1.330	1.330	1.330	1.330	1.330
Protein nitrogen		1.061	0.322	0.332	0.390	0.390	0.424	0.547	0.670	1.051
Nonprotein nitrogen		0.269	1.008	1.008	0.940	0.940	0.906	0.783	0.660	0.279
Polypeptid nitrogen		0.211	0.916	0.907	0.844	0.847	0.812	0.700	0.575	0.222
Amino nitrogen		0.040	0.073	0.083	0.078	0.075	0.077	0.066	0.067	0.040
Ammonia nitrogen		0.018	0.019	0.018	0.018	0.018	0.017	0.017	0.018	0.017
Total nitrogen	7	1.330	1.330	1.330	1.330	1.330	1.330	1.330	1.330	1.330
Protein nitrogen		1.061	0.367	0.390	0.333	0.547	0.524	0.524	0.524	0.983
Nonprotein nitrogen		0.269	0.963	0.940	0.997	0.883	0.806	0.806	0.806	0.347
Polypeptid nitrogen		0.205	0.861	0.832	0.884	0.778	0.706	0.706	0.708	0.279
Amino nitrogen		0.039	0.076	0.082	0.086	0.081	0.076	0.075	0.073	0.043
Ammonia nitrogen		0.025	0.026	0.026	0.027	0.024	0.024	0.025	0.025	0.025
Total nitrogen	14	1.330	1.330	1.330	1.330	1.330	1.330	1.330	1.330	1.330
Protein nitrogen		1.061	0.413	0.547	0.402	0.402	0.367	0.379	0.469	
Nonprotein nitrogen		0.269	0.917	0.883	0.928	0.928	0.963	0.951	0.861	
Polypeptid nitrogen		0.191	0.821	0.738	0.820	0.826	0.854	0.847	0.750	
Amino nitrogen		0.049	0.067	0.070	0.078	0.073	0.082	0.076	0.082	
Ammonia nitrogen		0.029	0.029	0.030	0.030	0.029	0.027	0.028	0.029	

An answer to this question was sought for, using the carbol-gelatin method previously described by Kendall and Walker.[6] Briefly, the method was to add 5 c c of culture of B. proteus at the intervals stated to 95 c c of a solution of gelatin in 0.5% phenol (carbolic acid)

solution. Two samples were prepared simultaneously from each of the cultures of a particular date. One of these was analyzed at once in accordance with the nitrogenous constituents tabulated. This was the control. The other sample was incubated 72 hours at 37 C. to allow the enzyme to act. The same determinations were then made.[16]

DISCUSSION OF TABLE 2

Two distinctive features stand out clearly in carbol gelatin inoculated from the mediums containing the smaller amounts of glucose, namely, the rapid reduction in the protein-nitrogen fraction, indicating a cleavage of the gelatin protein molecule by a soluble enzyme, and also no appreciable change in the ammonia nitrogen. The former is a measure of the activity of the soluble proteolytic enzyme. The latter shows distinctly that the deaminizing process depends on the presence of living bacteria. This is in harmony with the theory advanced by Kendall and Walker [6] that deamination is a measure of the intracellular utilization of protein or protein derivatives for energy by bacteria.

Little change is noticed in the amino nitrogen fraction; the greatest change amounts to less than 4% of the total nitrogen of the medium.

The great change is between the protein nitrogen and the "polypeptid" nitrogen. The former is reduced from 54% to less than 8% in the first few days (plain gelatin culture; very little change is noted in the 1.5 glucose culture). During the first 24 hours of growth the change amounts to rather more than 20%.[17] On the other hand, the polypeptid nitrogen rises from less than 13% to more than 59% in the same time. In this respect the protein-nitrogen and polypeptid-nitrogen fractions of the carbol gelatin follow closely those observed in the cultures containing living bacteria. It would appear that the significant change, therefore, is due to the activity of the soluble enzyme. The absence of deamination in the carbol gelatin, on the contrary, is in striking contrast to the rapid deamination in the plain gelatin culture, and the mediums containing the lower percentages of glucose.

[16] The table (table 2) shows only one control for each day. A separate control for each sample was made, because the amounts of ammonia, protein nitrogen, and polypeptid nitrogen varied somewhat with the culture used. To save space, all controls are reduced to that of the plain gelatin culture, and the results of incubation reduced accordingly. It was found necessary on the fourth day to increase the gelatin to 10%. This explains the apparently abrupt change in total nitrogen and other nitrogenous constituents on this date in comparison with the preceding days.

[17] This unequivocal result would appear to controvert Berman and Rettger's unsupported surmise that "a bacterial proteolytic enzyme, as a rule, is not produced within twenty-four hours," etc.

It is quite clear that the methods employed [11] offer a much more complete study of the nitrogen spectrum, both of cultures and of enzymes, than those previously utilized by Berman and Rettger. These methods, furthermore, appear to be applicable to the study of the action of enzymes, such as pepsin and trypsin, as well as bacterial enzymes, on nitrogenous substrates.

CONCLUSIONS

These quantitative measurements of the nitrogenous metabolism of B. proteus in various mediums, and of the nature and extent of the soluble enzyme of B. proteus, confirm and extend the observations of Kendall and Walker, referred to in the foregoing, in the following particulars, which were specifically studied:

"Bacillus proteus forms a soluble proteolytic enzyme in plain gelatin. The mature enzyme may be obtained in an active state freed from bacteria."

"The enzyme appears to be a preparatory enzyme in the sense that it prepares protein for assimilation by the bacteria; it has no demonstrable rôle in the intracellular utilization of the protein by the bacteria."

"The liquefaction of gelatin by the bacteria-free enzyme is not accompanied by the liberation of ammonia; deamination is an independent phenomenon associated with the intracellular utilization of the products of proteolysis by the organisms themselves."